MPTP: An Industrial Chemical and Contaminant of Illicit Narcotics Stimulates a New Era in Research on Parkinson's Disease

by I. J. Kopin*

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) causes selective destruction of dopaminergic neurons of the nigrostriatal pathway in humans and other primates. It is less specific and much less potent in mice and has only slight effects in rats. Differences in rates and sites of metabolism of MPTP to its active, toxic, highly polar metabolite, MPP^+ (1-methyl-4-phenylpyridine), appear to influence species specificity. In rats, type B monoamine oxidase (MAO-B), which mediates the conversion of MPTP to MPP^+, may act as an enzymatic barrier at brain microvessels, whereas in primates the enzyme, present mainly in astrocytes, appears important for bioactivation of MPTP into the toxic metabolite. MPP^+ is a substrate for catecholamine uptake sites and is concentrated in these neurons. The molecular mechanism of MPP^+ toxicity has not been established definitively, but conversion to a free radical or uptake by mitochondria and inhibition of mitochondrial respiratory enzymes, leading to calcium release and cell death have been suggested. The discovery of toxin which causes an animal model of Parkinson's disease has stimulated new research on environmental factors that might contribute to this progressive degenerative disorder and provides a means for assessing new approaches to therapy.

Introduction

It was recently discovered that drug abusers who accidentally self-administered 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) developed, over a period of a few days, a severe motor disorder which closely resembles an advanced stage of Parkinson's disease (1,2). MPTP is formed (Fig. 1) by the dehydration of an intermediate compound required for the synthesis of an active narcotic compound, 1-methyl-4-propionoxypyridine (MPPP), an analogue of meperidine. The toxicity of MPTP was not recognized until the occurrence of the parkinsonian syndrome among these drug abusers, but it is now recognized that several chemists who had synthesized large quantities of MPTP as an industrial intermediate developed parkinsonism as a consequence of exposure to this toxin (3,4). Studies in primates have shown that the brain lesions, biochemical alterations, and motor abnormalities found after MPTP administration resemble closely those found in spontaneous Parkinson's disease (5–7). Duvoisin (8) has summarized the epidemiological evidence that Parkinson's disease is not likely to be a heritable disorder. This evidence that environmental factors are important and the discovery that exposure to a chemical substance can produce neurological deficits almost identical to those of Parkinson's disease has excited new efforts at understanding the actions of MPTP and identifying environmental factors that might prevent or alleviate the progression of the disease.

Mechanisms Influencing MPTP Toxicity

A number of different, sometimes competing, processes influence the ability of a toxin to attack and destroy specific cells. Metabolism of the administered agent may be responsible for bioactivation and/or detoxification of the compound or its metabolite; the relative rates of activation and detoxification may determine vulnerability. Diffusion or active transport of the compound or its metabolite may limit or enhance toxicity by determining at which sites the toxin can reach sufficiently high concentrations to interfere with vital cellular processes. Cellular constituents may provide defenses against the toxic effects or may facilitate the actions of the toxin and thereby influence cellular vulnerability. The capacity of the cell to repair or replace damaged organelles or enzymes can also be critical in determining cell survival after a toxic insult. Many of these factors appear to influence significantly MPTP toxicity and may account for differences in species vul-

*National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892.
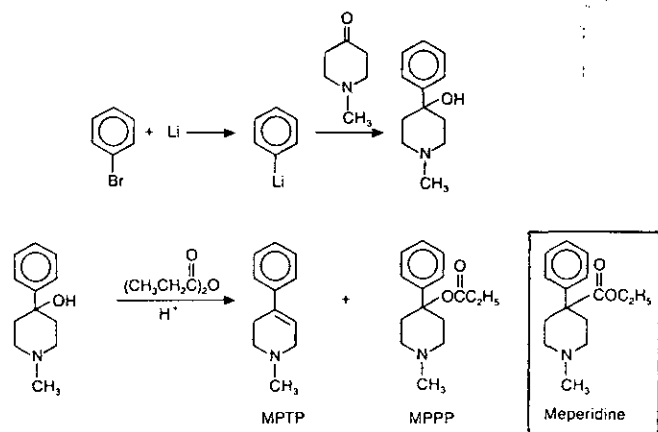
FIGURE 1. Chemical synthesis of the meperidine analogue, MPPP, and the side reaction product, MPTP.

FIGURE 2. Metabolism of MPTP to MPP⁺.

nerability as well as tissue specificity of the toxin. Low doses of MPTP cause relatively specific damage to the dopaminergic neurons in the nigrostriatal pathway of primates and also are effective in dogs; larger doses are required to affect mice, but specificity is lost in many dopaminergic systems and even the noradrenergic neurons are affected. Other rodents (rats, guinea pigs) appear almost invulnerable to the systemically administered toxin.

Metabolism of MPTP to MPP⁺

MPTP is a fat-soluble compound that is absorbed rapidly and freely penetrates membranes, including the blood-brain barrier of mice and monkeys (9). Shortly after administration of MPTP to monkeys, however, it is almost completely converted to its quarternary derivative, 1-methyl-4-phenyl-pyridine (MPP⁺), which is formed in all tissues, including the brain (9,10). MPP⁺ persists in the brain of monkeys for many days (the half-life has been estimated to be about 10 days), but rapidly disappears from mouse brain (9). Enzymatic conversion of MPTP to MPP⁺ was first demonstrated in rat (11) and monkey (12) brain homogenates, but has subsequently been found to occur in homogenates or slices of many tissues. Since formation of MPP⁺ is mediated by mitochondrial enzymes and is inhibited by drugs that block type B monoamine oxidase (MAO-B) but not by those that block only type A MAO (MAO-A), it is likely that MAO-B is responsible for the oxidation of MPTP (11). The oxidation appears to occur in two steps, with 1-methyl-4-phenyl-3,4,-dihydropyridine (MPDP⁺) as an intermediate (Fig. 2). Conversion of MPDP⁺ to MPP⁺ may proceed by spontaneous auto-oxidation or by the action of MAO.

Formation of MPP⁺ is essential to MPTP toxicity since inhibition of MAO-B, which blocks formation of MPP⁺ and even elevates brain levels of MPTP (9), prevents the dopamine neuron-damaging effects (9,13,14). The protective effect of MAO-B inhibitors has been repeatedly demonstrated, in tissue cultures as well as in
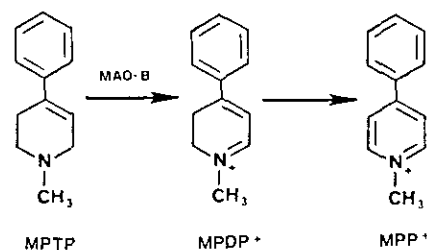
vivo. The acute behavioral effects of MPTP, however, do not appear to be the result of MPP⁺ formation because they are unaffected by pretreatment with MAO-B inhibitors.

The localization of MAO-B may be an important determinant of MPTP toxicity. MAO-B is not the major form of MAO in dopaminergic neurons, but does predominate in astrocytes and serotonergic neurons (15) as well as in blood vessel walls of some species. After IV administration of ³H-MPTP, the distribution of radioactivity in brain resembles that of ³H-pargyline (16,17), an irreversible MAO inhibitor. If MAO-B is present in sufficiently high concentrations in capillary or blood vessel walls outside the blood-brain barrier, conversion of MPTP to MPP⁺ at this site might limit entry of the toxin into the brain, as MPP⁺ does not readily enter brain. One day after ³H-MPTP administration to a monkey, Markey et al. (9) found that arterial walls accumulated the radiolabel as densely as did the locus coeruleus, lateral parabrachial nucleus, and neostriatum. In other species, vascular MAO-B may predominate. Harik et al. (18) attribute the resistance of rats to the toxic effects of systemically administered MPTP to an enzymatic barrier resulting from the high levels of MAO-B in the capillaries of rat brain. They showed that ³H-pargyline binding to rat brain microvessels was 10-fold higher than binding to microvessels from human or mouse brain and that most of the tritum was bound to MAO-B. Furthermore, MPTP oxidation by rat cerebral microvessels was 30-fold more rapid than by human microvessels; mouse microvessels were intermediate in MPTP-oxidizing capacity. The relative activities of MAO-B in astrocytes or serotonergic neurons inside, and capillary endothelium outside, of the blood-brain barrier might regulate the toxicity of MPTP administered directly into the brain, as well as by IV injection. Thus, while MPP⁺ administered into the neostriatum of rats is toxic, MPTP injected in the same dose is not (19). Although MPTP is toxic to dopaminergic neurons in explants of rat embryo mesencephalon (20), it is not toxic when infused directly into the substantia nigra (21).

Selective Uptake of MPP⁺ by Catecholaminergic Neurons

While MAO-B in astrocytes and serotonergic neurons may be responsible for the conversion of MPTP to

MPP$^+$ in brain, this does not explain the specificity of MPTP toxicity for dopaminergic neurons. MPP$^+$, which is a quartenary amine, diffuses with difficulty across lipid membranes, but studies with astrocytes in tissue culture have suggested that this compound can escape into the surrounding media. MPP$^+$ is taken up by synaptosomes prepared from rat striatum or cortex; inhibition of this uptake by various drugs indicates that the uptake process for dopamine in the striatal synaptosomes and for norepinephrine in cortical synaptosomes is responsible for MPP$^+$ accumulation (22). Pharmacological inhibition of dopamine uptake blocks the neurotoxic effects of MPTP in mice (23–25). Blockade by dopamine uptake inhibition of the toxic effects of MPTP in monkeys has also been demonstrated, but protection required administration of the inhibitor for weeks after MPTP (26).

Uptake of MPP$^+$ by catecholaminergic neurons adequately explains the selectivity of the toxic effects in mice. In this species, noradrenergic as well as most dopaminergic neurons are affected, but the amine depletion is often transient and few cells appear to have been killed. There are wide variations in the vulnerability of catecholaminergic neurons in mouse brain to MPTP toxicity. Dopaminergic neurons in C57BL/6 mice were found to be more sensitive to MPTP than were those of NMRI or CBA/Ca mice and those of Swiss-Webster mice least sensitive (27), but in C57BL/6 mice, norepinephrine in the frontal cortex was not significantly altered, whereas in the other strains there were 50 to 89% decreases in cortical norepinephrine. In another study, white BKW mice were as sensitive as black C57BL/6 mice to MPTP depletion of dopamine (28), whereas noradrenergic neurons appeared least vulnerable. Differences in vulnerability among Swiss-Webster mice obtained from different supplies has also been noted (29). Age, route of MPTP administration, and sex have also been cited as variables in determining toxicity of MPTP in monkeys as well as mice. Thus, while uptake of MPP$^+$ into dopaminergic neurons appears to be necessary for MPTP toxicity, this does not explain completely the neuronal specificity.

Binding of MPP$^+$ to Neuromelanin

Neuromelanin is the pigmented substance that is responsible for the dark color of the substantia nigra in primates and several other species. It is formed from oxidation of dopamine (or other catechols) to quinones which polymerize and are deposited on lipofuscin. Neuromelanin accumulates with age and is generally regarded as a waste product. The association between neuromelanin content in primates and dogs and the relative vulnerability of nigrostriatal neurons to MPTP in these species contrasted with the almost complete absence of neuromelanin in rodents, which are much less vulnerable to the toxin. The parallel increase of neuromelanin and vulnerability to MPTP with age in primates suggested that neuromelanin might participate in the toxic mechanism or its presence was indicative

of a lack of cellular defense mechanisms to protect against damage. Vulnerability to MPTP, however, also increases with age in mice (30) that lack brain neuromelanin. Neuromelanin binds MPP$^+$ with relatively high affinity (31), and it has been suggested that these granules may store the MPP$^+$. Slow release of MPP$^+$ into the cytoplasm from this depot might maintain toxic levels in the cytoplasm sufficiently long to cause irreversible cell damage and death. Chloroquin, which blocks binding of MPTP to neuromelanin, appears to decrease MPTP toxicity (32). Studies with radiolabeled MPTP, however, show that the toxin accumulates only in the cell terminals, not in the cell bodies where neuromelanin is located.

Mechanisms of Cellular Toxicity of MPP$^+$

The accumulation and persistence of MPP$^+$ at nerve terminals appears to result in damage to the neuron and may, in vulnerable cells, cause neuronal death. Several hypotheses have been proposed to explain the molecular mechanisms responsible for MPP$^+$ toxicity. One group of hypotheses suggests that free radical formation, which can result in production of superoxides, hydrogen peroxide, hydroxyl free radicals, and a limited capacity to protect against oxidative stress, is involved in MPP$^+$ toxicity. Normally superoxide is converted to hydrogen peroxide by superoxide dismutase and the hydrogen peroxide is converted to water and oxygen by catalase. If these reactions do not proceed sufficiently rapidly to remove superoxide or hydrogen peroxide, hydroxyl free radicals can form. These free radicals can attack membranes, organelles, DNA or RNA, carbohydrates, or proteins, resulting in inactivation of vital enzymes. Superoxide dismutase, catalase, glutathione peroxidase, and other soluble reducing substances protect the cell from accumulation of excess free radicals, but when free radical formation is rapid and reductive agents are depleted, these protective mechanisms may be inadequate.

The evidence for a role of redox cycling and free radical involvement in MPP$^+$ toxicity has been largely indirect. Although Sinha et al. (33) could not demonstrate by electron spin resonance free radical formation from MPP$^+$ under conditions in which paraquat free radicals were apparent, by using a spin-trapping technique, they did find significant stimulation by MPP$^+$ of superoxide and hydroxyl radical formation. Formation of these free radicals occurred during incubation of NADPH cytochrome P-450 reductase with NADPH in the presence of oxygen, but was less rapid than when paraquat was used.

Diethyl dithiocarbamate (DCC), which chelates copper and inhibits superoxide dismutase, potentiates the toxicity of both paraquat (34) and MPTP (35) administered to mice. DCC, however, also inhibits other copper-dependent enzymes, including aldehyde dehydrogenase. Ethanol, which is a good hydroxyl radical scavenger, inhibits MPP$^+$ potentiation of free radical formation *in vitro* (33), but ethanol and its metabolite,

acetaldehyde, potentiate the toxicity of MPTP in mice (36), possibly by inhibiting superoxide dismutase.

Like paraquat, when administered systemically, MPP^+ causes severe toxic damage in the lungs and increases plasma levels of glutathione disulfide, an indication of oxidative stress (37). In isolated hepatocytes, both MPP^+ and paraquat rapidly deplete ATP and in the presence of BCNU, an inhibitor of glutathione reductase, decrease glutathione levels (38). Glutathione depletion by MPTP (39,40) does not appear to be a major factor in its toxicity since agents that elevate glutathione tissue levels fail to diminish MPP^+ toxicity (41).

Attempts to prevent or diminish the toxicity of MPTP by treatment with one or more of several antioxidants administered to mice before, during, and after the toxin have not been consistently successful. By treatment with α-tocopherol (2.5 g/kg), β-carotene (100 mg/kg), 1-ascorbic acid (100 mg/kg), or N-acetylcysteine (50 mg/kg) daily for 5 days beginning 2 days before administration of the toxin, Perry et al. (42) partially prevented the depletion of striatal dopamine 1 month after the administration of a single dose of MPTP (40 mg/kg). Using similar methods, however, neither Baldessarini et al. (43) nor Martinovits et al. (44) could demonstrate any protection from the toxicity at 7 or 30 days.

Inhibition by MPP^+ of Mitochondrial Enzymes

Since interference with essential mitochondrial functions could lead to cell death, effects of MPTP and its metabolites on mitochondrial function became of interest.

In mitochondria isolated from rat or mouse brain, MPP^+ interferes with NADH-linked oxidation of pyruvate or glutamate without affecting the oxidation of succinate. This suggests that the site of interaction with the respiratory process is at complex I (45). In slices of mouse neostriatum, MPP^+ or MPTP increase lactate formation and enhance accumulation of glutamate and aspartate, consistent with inhibition of mitochondrial oxidation. These effects of MPTP are prevented by pretreatment with pargyline, indicating that the toxic effects are dependent on formation of MPP^+ (46). This suggests reversible inhibition by MPP^+, but not by MPTP, of NADH dehydrogenase cytochrome c reductase (47).

Ramsay et al. (48–50) and Frei and Richter (51) showed that MPP^+ is accumulated by an energy-dependent uptake process into intact mitochondria. The uptake process appears to be dependent on the electrochemical gradient of the mitochondrial membrane, as valinomycin and K^+, which collapse the gradient, abolish MPP^+ uptake. Mitochondrial uptake of MPP^+ is not inhibited by dopamine uptake inhibitors (e.g., mazindole), but is blocked by respiratory enzyme inhibitors and uncouplers. MPP^+ blockade of mitochondrial respiration is far less efficient in inverted mitochondria or in isolated mitochondrial inner membranes than in intact mitochondria (50). This is consistent with the view that high concentrations of MPP^+ are attained in intact mitochondria as a result of its energy-dependent uptake by intact mitochondria (49). Since calcium ions interfere with the uptake of MPP^+ by isolated rat mitochondria (51), it was thought that the ions might compete for the same electrochemical gradient. Although neither 6-OH-DA nor MPP^+ separately have much effect on the rate of release of calcium sequestered in mitochondria, together they greatly increase the rate of calcium efflux. MPTP inhibits calcium efflux and almost completely prevents the enhanced calcium release attending combined MPP^+-6-OH-DA treatment. Since excess calcium release has been implicated as a cause of cell death, it was suggested that the toxic effects of MPTP might be mediated through release of calcium from mitochondria.

With glutamate or malate as substrates, the respiratory rate of mitochondria from brains of 24-month-old rats was lower than that of mitochondria from brains of 2-month-old animals, and glutamate uptake was markedly lower (52,53). There was no difference, however, when succinate was used as substrate. The rate of calcium entry in mitochondria from aged rats is also lower than the rate in mitochondria from young rats. It is perhaps not fortuitous that aging produces changes in mitochondrial respiration and calcium distribution similar to the effects of MPP^+ and that MPTP toxicity increases with age.

Summary of Mechanisms Involved in MPTP Toxicity

A schematic representation of the processes involved in MPTP toxicity is shown in Figure 3. It is clear that MPTP itself is not toxic and must be bioactivated by conversion to MPP^+. This is mediated by MAO-B. MPP^+, however, is a very polar compound and does not penetrate into the brain, but MPTP can easily diffuse across the blood-brain barrier to reach astrocytes (or other cells) that contain MAO-B. If formation of MPP^+ from MPTP in the endothelial cells is rapid, these cells can act as an enzymatic barrier to entry of MPTP into the brain tissue. Measurements of total brain MPP^+ would include the MPP^+ in the endothelial cells, but it might be expected that the MPP^+ in these cells would be lost rapidly. It has been proposed that high levels of endothelial MAO-B protect rats from MPTP toxicity. Once in the brain, MPTP is converted to MPP^+ in astrocytes and the quarternary amine taken up into catecholaminergic neurons. The molecular mechanisms responsible for MPP^+ toxicity have not been definitively established, but interactions with neuromelanin, possible free-radical formation, or concentration in mitochondria with consequent blockade of oxidative enzymes have been suggested. The wide variability of regional vulnerability in monkey species, mice strains, and even among humans exposed to MPTP, in addition to differences in the neurons themselves, suggests that several of these processes may act in concert to determine the levels of MPP^+ to which neurons are exposed.
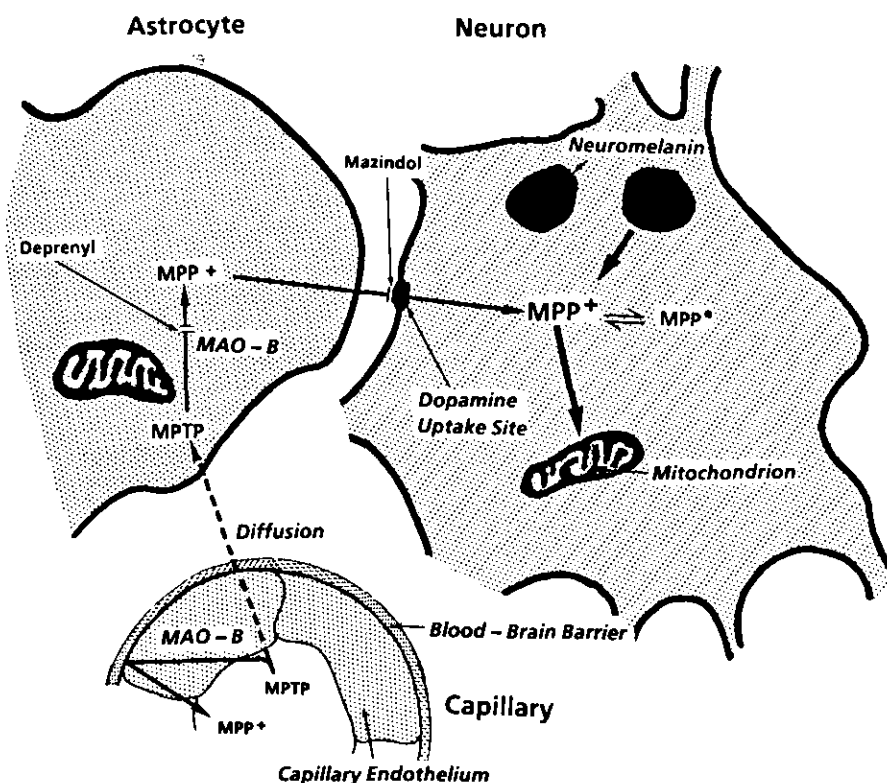
FIGURE 3. Schematic representation of the processes involved in MPTP toxicity. MAO-B in the capillary endothelium may serve as an enzymatic barrier to entry of MPTP by converting it to MPP$^+$, which cannot penetrate the blood-brain barrier. If MPTP diffuses into the brain, it can be metabolized by astrocyte (or serotoninergic neuronal) MAO-B to MPP$^+$. When MPTP leaves the astrocyte, the specific dopamine uptake mechanism can concentrate the toxin in the cytoplasm. The mechanisms of toxicity may involve neuromelanin storage and release of MPP$^+$, free radical formation, or direct attack on mitochondrial oxidative enzymes (see text).

Impact of the Discovery of MPTP Toxicity on Parkinson's Disease Research

The realization that nigrostriatal dopaminergic neurons are particularly vulnerable to a toxin and that the relative specific damage to this system can produce all the cardinal signs and symptoms of Parkinson's disease has stimulated new hypotheses regarding the pathogenesis of this disorder. As indicated above, Parkinson's disease is normally not a heritable disorder. Stimulated by the hypothesis that an environmental toxin may be responsible for the development of Parkinson's disease, a number of investigators have sought to identify other compounds that may produce similar toxic changes. The vulnerability of mice to MPTP toxicity has provided a useful means of screening such compounds (54). It is evident, however, that in order for these analogues to be active, they must be able to penetrate into brain, be converted to a toxic analogue of MPP$^+$ which is a substrate for the dopamine uptake site and, after entry into the neuron, interfere with a vital process. Several such analogues of MPTP have been found (e.g., 2'-methyl-MPTP, 4'-fluoro-MPTP, 4'-amino-MPTP), but as might

be expected, there are species differences in vulnerability to these toxins. It is clear, however, that the N-methyl group, the unsaturated pyridine ring, and the 4-aromatic ring are necessary for the toxic effects. It has been proposed that the enzymatic N-methylation might bioactivate a molecule lacking this group (53), expanding the possible number of substances that might be converted to a specific dopaminergic neuronal toxin.

The ability of inhibitors of MAO-B to block the toxicity of MPTP and the report by Birkmayer et al. (56) that deprenyl, a specific MAO-B inhibitor, not only potentiates the therapeutic efficacy of L-Dopa but prolongs life expectancy of patients with Parkinson's disease, stimulated further speculation on the role of MAO-B in bioactivation of an environmental agent to produce a toxin that is responsible for the degenerative process responsible for progression of the disease. Indeed, clinical trials designed to examine the influence of deprenyl in arresting or slowing the progression of Parkinson's disease have been instituted.

The availability of an animal model that faithfully emulates the spontaneous human motor disorder provides a useful test system with which to evaluate new therapeutic agents. By infusing MPTP into a carotid artery, monkeys have been made hemiparkinsonian (57). These

animals are able to feed themselves and survive well without treatment with L-dopa or dopamine agonists. Spontaneous motor activity consistently results in turning toward the lesioned side, but when dopamine agonists are administered, the direction of turning is reversed as a result of stimulation of supersensitive receptors on the lesioned side. The rate and duration of the reversed turning are dose dependent and provide a means for quantification of drug efficacy.

Both hemiparkinsonism and fully parkinsonian animals have been subjected to tissue transplants using adrenal medulla or fetal mesencephalon cells. These transplants survive, produce dopamine, may stimulate additional sprouting of surviving dopaminergic neurons (e.g., from the ventral area), and result in functional recovery. The improvement is transient with adrenal medullary tissue, but fetal tissue transplants appear to survive for at least half a year (58).

Thus, as the result of the tragic and dramatic accidental discovery of the toxicity of MPTP, new studies of the pathogenesis and treatments of Parkinson's disease have been stimulated and improve prospects for preventing, arresting, or even reversing the progression of the degenerative processes responsible for neuronal cell death.

REFERENCES

1. Davis, G. C., Williams, A. C., Markey, S. P., Ebert, M. H., Caine, E. D., Riechert, C. M., and Kopin, I. J. Chronic parkinsonism secondary to intravenous injection of meperidine analogues. Psychiatry Res. 1: 249–54 (1979).
2. Langston, J. W., Ballard, P., Tetrud, J. W., and Irwin, I. Chronic parkinsonism in humans due to a product of meperidine-analog synthesis. Science 219: 979–980 (1983).
3. Sahgal, A., Andrews, J. S., Biggins, J. A., Candy, J. M., Edwardson, J. A., Keith, A. B., Turner, J. D., and Wright, C. N-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) affects locomotor activity without producing a nigrostriatal lesion in the rat. Neurosci. Lett. 48: 179–84 (1984).
4. Burns, R. S., LeWitt, P. A., Ebert, M. H., Pakkenberg, H., and Kopin, I. J. The clinical syndrome of striatal dopamine deficiency. Parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). N. Engl. J. Med. 312: 1418–1421 (1985).
5. Burns, R. S., Chiueh, C. C., Markey, S. P., Ebert, M. H., Jacobowitz, D. M., and Kopin, I. J. A primate model of parkinsonism: Selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Proc. Natl. Acad. Sci. (U.S.) 80: 4546–4550 (1983).
6. Langston, J. W., and Ballard, P. Parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): Implications for treatment and the pathogenesis of Parkinson's disease. Can. J. Neurol. Sci. 11: 160–165 (1984).
7. Jenner, P., Rupniak, N. M., Rose, S., Kelly, E., Kilpatrick, G., Lees, A., and Marsden, C. D. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism in the common marmoset. Neurosci. Lett. 50: 85–90 (1984).
8. Duvoisin, R. C. Parkinson's disease acquired or inherited? Can. J. Neurol. Sci. 11: 151–155 (1984).
9. Markey, S. P., Johannessen, J. N., Chiueh, C. C., Burns, R. S., and Herkenham, M. A. Intraneuronal generation of a pyridinium metabolite may cause drug-induced parkinsonism. Nature 311: 464–467 (1984).
10. Langston, J. W., Irwin, I., Langston, E. B., and Forno, L. S. 1-Methyl-4-phenylpyridinium ion (MPP$^+$): Identification of a metabolite of MPTP, a toxin selective to the substantia nigra. Neurosci. Lett. 48: 87–92 (1984).
11. Chiba, K., Trevor, A., and Castagnoli, N., Jr. Metabolism of the neurotoxic tertiary amine, MPTP, by brain monoamine oxidase. Biochem. Biophys. Res. Commun. 120: 574–578 (1984).
12. Irwin, I., and Langston, J. W. Selective accumulation of MPP$^+$ in the substantia nigra: A key to neurotoxicity? Life Sci. 36: 207–212 (1985).
13. Langston, J. W., Irwin, I., Langston, E. B., and Forno, L. S. Pargyline prevents MPTP-induced parkinsonism in primates. Science 225: 1480–1482 (1984).
14. Heikkila, R. E., Manzino, L., Cabbat, F. S., and Duvoisin, R. C. Protection against the dopaminergic neurotoxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine by monoamine oxidase inhibitors. Nature 311: 467–469 (1984).
15. Westlund, K. N., Denney, R. M., Kochersperger, L. M., Rose, R. M., and Abell, C. W. Distinct monoamine oxidase A and B populations in primate brain. Science 230: 181–183 (1985).
16. Parsons, B., and Rainbow, T. C. High-affinity binding sites for [^{3}H]MPTP may correspond to monoamine oxidase. Eur. J. Pharmacol. 102: 375–377 (1984).
17. Rainbow, T. C., Parsons, B., Wieczorek, C. M., and Manaker, S. Localization in rat brain of binding sites for parkinsonian toxin MPTP: Similarities with [^{3}H]pargyine binding to monoamine oxidase. Brain Res. 330: 337–342 (1985).
18. Harik, S. I., Mitchell, M. J., and Kalaria, R. N. Human susceptibility and rat resistance to systemic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxicity correlate with blood brain barrier monoamine oxidase B activity. Neurology 37: 338–339 (1987).
19. Bradbury, A. J., Costall, B., Domeney, A. M., Jenner, P. J., Marsden, C. D., and Naylor, R. J. The neurotoxic actions of 6-hydroxydopamine infused into the rat substantia nigra. Neurosci. Lett. 66: 208–212 (1986).
20. Mytilineou, C., and Cohen, G. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine destroys dopamine neurons in explants of rat embryo mesencephalon. Science 225: 529–531 (1984).
21. Chiueh, C. C., Markey, S. P., Burns, R. S., Johannessen, J. N., Pert, A., and Kopin, I. J. Neurochemical and behavioral effects of systemic and intranigral administration of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in the rat. Eur. J. Pharmacol. 100: 189–194 (1984).
22. Javitch, J. A., and Snyder, S. H. Uptake of MPP($^+$) by dopamine neurons explains selectivity or parkinsonism-inducing neurotoxin, MPTP. Eur. J. Pharmacol. 13: 455–456 (1984).
23. Sundström, E., and Jonsson, G. Pharmacological interference with the neurotoxic action of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on central catecholamine neurons in the mouse. Eur. J. Pharmacol. 110: 293–299 (1985).
24. Pileblad, E., and Carlsson, A. Catecholamine-uptake inhibitors prevent the neurotoxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mouse brain. Neuropharmacology 24: 689–692 (1985).
25. Ricaurte, G. A., Langston, J. W., DeLanney, L. E., Irwin, I., and Brooks, J. D. Dopamine uptake blockers protect against the dopamine depleting effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in the mouse striatum. Neurosci. Lett. 59: 259–264 (1985).
26. Schultz, W., Scarnati, E., Sundström, E., Tsutsumi, T., and Jonsson, G. The catecholamine uptake blocker nomifensine protects against MPTP-induced parkinsonism in monkeys. Exp. Brain Res. 63: 216–220 (1986).
27. Sundström, E., Strömberg, I., Tsutsumi, T., Olson, L., and Jonsson, G. Studies on the effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on central catecholamine neurones in C57 BL/6 mice. Comparison with three other strains of mice. Brain Res. 405: 36–38 (1987).
28. Bradbury, A. J., Costall, B., Jenner, P. G., Kelly, M. E., Marsden, C. D., and Naylor, R. J. The effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on striatal and limbic catecholamine neurones in white and black mice. Antagonism by monoamine oxidase inhibitors. Neuropharmacology 25: 897–904 (1986).

29. Heikkila, R. E. Differential neurotoxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in Swiss-Webster mice from different sources. Eur. J. Pharmacol. 119: 127–128 (1985).

30. Jarvis, M. F., and Wagner, G. C. Age-dependent effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Neuropharmacology 24: 581–583 (1985).

31. D'Amato, R. J., Lipman, Z. P., and Snyder, S. H. Selectivity of parkinsonian neurotoxin MPTP: Toxic metabolite MPP$^+$ binds to neuromelanin. Science 231: 987–989 (1986).

32. D'Amato, R. J., Alexander, G. M., Schwartzman, R. G., Kitt, C. A., Price, D. L., and Snyder, S. H. Neuromelanin: A role in MPTP-induced neurotoxicity. Life Sci. 40: 705–712 (1987).

33. Sinha, B. K., Singh, Y., and Krishna, G. Formation of superoxide and hydroxyl radicals from 1-methyl-4-phenylpyridinium ion (MPP$^+$): Reductive activation by NADPH cytochrome P-450 reductase. Biochem. Biophys. Res. Commun. 135: 583–588 (1986).

34. Goldstein, B. D., Rozen, M. G., Quintavalla, J. C., and Amoruso, M. A. Decrease in mouse lung and liver glutathione peroxidase activity and potentiation of the lethal effects of ozone and paraquat by the superoxide dismutase inhibitor diethyl-dithiocarbamate. Biochem. Pharmacol. 29: 27–30 (1979).

35. Corsini, G. U., Pintus, S., Chiueh, C. C., Weiss, J. F., and Kopin, I. J. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxicity in mice is enhanced by pretreatment with diethyldithiocarbamate. Eur. J. Pharmacol. 119: 127–128 (1986).

36. Corsini, G. U., Zuddas, A., Bonuccelli, U., Schinelli, S., and Kopin, I. J. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxicity in mice is enhanced by ethanol or acetaldehyde. Life Sci. 40: 827–832 (1987).

37. Johannessen, J. N., Adams, J. D., Schuller, H. M., Bacon, J. P., and Markey, S. P. 1-Methyl-4-phenylpyridine (MPP$^+$) induces oxidative stress in the rodent. Life Sci. 38: 743–749 (1986).

38. DiMonte, D., Sandy, M. S., Ekström, G., and Smith, M. T. Comparative studies on the mechanisms of paraquat and 1-methyl-4-phenylpyridine (MPP$^+$) cytotoxicity. Biochem. Biophys. Res. Commun. 137: 303–309 (1986).

39. Yong, V. W., Perry, T. L., and Krisman, A. A. Depletion of glutathione in brainstem of mice caused by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine is prevented by antioxidant pretreatment. Neurosci. Lett. 639: 56–60 (1986).

40. Ferraro, T. N., Golden, G. T., DeMattei, M., Hare, T. A., and Fariello, R. G. Effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on levels of glutathione in the extrapyramidal system of the mouse. Neuropharmacology 25: 1071–1074 (1986).

41. Perry, T. L., Yong, V. W., Jones, K., and Wright, J. M. Manipulation of glutathione contents fails to alter dopaminergic nigrostriatal neurotoxicity of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in the mouse. Neurosci. Lett. 70: 261–265 (1986).

42. Perry, T. L., Yong, V. W., Clavier, R. M., Jones, K., Wright, J. M., Foulks, J. G., and Wall, R. A. Partial protection from the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine by four different antioxidants in the mouse. Neurosci. Lett. 60: 109–114 (1985).

43. Baldessarini, R. J., Kula, N. S., Francoeur, D., and Finklesteine, S. P. Antioxidants fail to inhibit depletion of striatal dopamine by MPTP [letter]. Neurology 36: 735 (1986).

44. Martinovits, G., Melamed, E., Cohen, O., Rosenthal, J., and Uzzan, A. Systemic administration of antioxidants does not protect mice against the dopaminergic neurotoxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Neurosci. Lett. 69: 192–197 (1986).

45. Nicklas, W. J., Vyas, I., and Heikkila, R. E. Inhibition of NADH-linked oxidation in brain mitochondria by 1-methyl-4-phenylyridine, a metabolite of the neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Life Sci. 36: 2503–2508 (1985).

46. Vyas, I., Heikkila, R. E., and Nicklas, W. J. Studies on the neurotoxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine: Inhibition of NAD-linked substrate oxidation by its metabolite, 1-methyl-4-phenylpyridinium. J. Neurochem. 46: 1501–1507 (1986).

47. Poirier, J., and Barbeau, A. 1-Methyl-4-phenylpyridinium-induced inhibition of nicotinamide adenosine dinucleotide cytochrome C reductase. Neurosci. Lett. 62: 7–11 (1985).

48. Ramsay, R. R., Dadgar, J., Trevor, A., and Singer, T. P. Energy-driven uptake of N-methyl-4-phenylpyridine by brain mitochondria mediates the neurotoxicity of MPTP. Life Sci. 39: 581–588 (1986a).

49. Ramsay, R. R., Salach, J. I., and Singer, T. P. Uptake of the neurotoxin 1-methyl-4-phenylpyridine (MPP$^+$) by mitochondria and its relation to the inhibition of the mitochondrial oxidation of NAD$^+$-linked substrates by MPP$^+$. Biochem. Biophys. Res. Commun. 134: 743–748 (1986b).

50. Ramsay, R. R., and Singer, T. P. Energy-dependent uptake of N-methyl-4-phenylpyridinium, the neurotoxic metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, by mitochondria. J. Biol. Chem. 261: 7585–7587 (1986c).

51. Frei, B., and Richter, C. 1-methyl-4-phenylpyridine (MPP$^+$) together with 6-hydroxydopamine or dopamine stimulates Ca^{2+} release from mitochondria. FEBS Lett. 198: 99–102 (1986).

52. Vitorica, J., Clark, A., Machado., A., and Satrüstegui, J. Impairment of glutamate uptake and absence of alterations in the energy-transducing ability of old rat brain mitochondria. Mech. Ageing Dev. 29: 255–266 (1985).

53. Victorica, J., and Strüstegui, J. Involvement of mitochondria in the age-dependent decrease in calcium uptake of rat brain synaptosomes. Brain Res. 378: 36–48 (1986).

54. Youngster, S. K., Sonsacca, P. K., and Nevikkila, R. E. Evaluation of the biological activity of several analogues of the dopaminergic neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. J. Neurochem. 48: 929–934 (1987).

55. Ansher, S. S., Cadet, J. L., Jakoby, W. B., and Baker, J. K. Role of N-methyltransferases in the neurotoxicity associated with the metabolites of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and other 4-substituted pyridines present in the environment. Biochem. Pharmacol. 35: 3359–3363 (1986).

56. Birkmayer, W., Knoll, J., Riederer, P., Youdim, M. B., Hars, V., and Marton, J. Increased life expectancy resulting from addition of L-deprenyl to Madopar treatment in Parkinson's disease: A longterm study. J. Neural Transm. 64: 113–127 (1985).

57. Bankiewicz, K. S., Oldfield, E. H., Chiueh, C. C., Doppman, J. L., Jacobowitz, D. M., and Kopin, I. J. Hemiparkinsonism in monkeys after unilateral internal carotid artery infusion of MPTP. Life. Sci. 39: 7–16 (1986).

58. Bankiewicz, K. S., Plunkett, R., Oldfield, E. H., Jacobowitz, D. M., Porrino, L. J., Vaidya, U., DiPorzio, U., Schuette, W. H., Markowitz, A., London, W. T., and Kopin, I. J. Transient and long term functional improvement by adrenal and fetal mesencephalic implants into caudate nuclei of MPTP parkinsonian monkeys (abs.). Schmitt Neurological Sciences Symposium (1987), in press.